ORIGINAL ARTICLE

Evaluation of resuscitation fluids on endothelial glycocalyx, venular blood flow, and coagulation function after hemorrhagic shock in rats

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BACKGROUND: Endothelial glycocalyx (EG) plays an essential role in endothelium integrity and may be compromised by hemorrhagic shock. The effects of currently available resuscitation fluids such as Hextend (HEX) or lactated Ringer's solution (LR) on vascular function and coagulation are not well understood. The aim of the present study was to compare the effects of fresh frozen plasma (FFP) with HEX or LR in their ability to repair EG structure, promote volume expansion, increase blood flow, and prevent coagulopathy.

METHODS: A total of 121 microvessels from cremaster muscle were studied in 32 anesthetized instrumented rats. After baseline systemic and microvascular measurements, 40% hemorrhage followed by resuscitation was performed, and measurements were repeated. Coagulation was evaluated using ROTEM to assay clot formation time, clotting time, firmness, strength, and lysis. Velocity and "platelet component" of strength were calculated. Fluorescein isothiocyanate or Texas Red bound to Dextrans was injected to estimate EG thickness in vivo.

RESULTS: Respiratory rate, blood pH, base excess, and lactate returned to near-baseline levels in all treatments. Hemodilution caused by LR and HEX decreased firmness, prolonged clotting time, and lowered platelet counts. EG thickness in HEX- and LR-treated rats was 50% lower, and plasma syndecan 1 was 50% higher than sham and FFP groups. Blood flow and shear rate were restored in the HEX group. Resuscitation with FFP improved coagulation and blood flow.

CONCLUSION: Our findings support the concept of cardiovascular and microvascular stabilization by infused FFP, in which the increase in microvascular perfusion associated with restored EG is essential for an optimal resuscitation strategy. (*J Trauma Acute Care Surg*. 2013;75:759–766. Copyright © 2013 by Lippincott Williams & Wilkins)

KEY WORDS: Microcirculation; skeletal muscle; blood flow; coagulation; resuscitation.

Fluid resuscitation remains the cornerstone treatment of hemodynamically unstable patients. Resuscitation with standard of care asanguinous fluids may not improve patient outcomes and may interfere with the hemostatic system, affecting the coagulation cascade.[1–3] The Starling forces, which determine the movement of fluid across capillary membranes, are disrupted in shock, and asanguinous fluid from the interstitium is recruited into the vascular space in an attempt to compensate for the loss of volume. Colloids and crystalloids may exacerbate hemodilution and decrease oxygen delivery, impairing microvascular perfusion and endothelial function.[2,4,5]

The role of the endothelium has been intensively investigated during the last decade.[6,7] The endothelial glycocalyx (EG), an integral component of the endothelial barrier, is composed of proteoglycans such as syndecan 1 and glycosaminoglycans (GAGs). In addition to the GAGs and proteoglycans, plasma proteins are also essential constituents of the EG, demonstrated to be decreased by removing plasma proteins.[7–9] EG degradation may play a role in maintaining vascular integrity as suggested from studies in trauma and homeostasis,[10] ischemia/reperfusion,[11] and inflammation.[12–14] The EG may be one of the major determining factors of vascular homeostasis since degradation of its components, such as the syndecan 1, have been correlated with other tissue damage and coagulopathy biomarkers as well as mortality.[12] For instance, studies demonstrate that excessive sympathoadrenal and platelet activation can lead to an increase syndecan 1 levels in blood (possibly due to shedding).[10] Another possible mechanism of action involves antithrombin III, located on and within the EG. Heparin-like GAG modifies the action of several of these effector molecules such as thrombin, antithrombin III.[15] Shedding of proteoglycans and GAGs can enhance dramatically antithrombin III action, which is crucial for the potentiation of thrombin inhibition, leading to coagulopathy. Using intravital microscopy, we have obtained direct in vivo data showing glycocalyx thickness reduction in skeletal muscle and mesentery venules after hemorrhagic shock (HS).[5]

Considering these, it is important to simultaneously assess microcirculation, coagulation, and systemic parameters to elucidate the effects of colloids, crystalloid, and blood products to establish criteria for optimal resuscitation. This is the first article that uses this integrated approach, studying skeletal muscle venules. We hypothesized that small-volume resuscitation with fresh frozen plasma (FFP) after a moderate HS will protect the

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endothelial cell better than the standard of care lactated Ringer's solution (LR) and Hextend (HEX), by repairing the EG structure, improving blood flow, providing volume expansion, and preserving coagulation status. This hypothesis was tested by comparing the effects of resuscitation with FFP to that with standard of care fluids, LR and HEX, on EG, microvascular hemodynamics, coagulation, and systemic parameters.

MATERIALS AND METHODS

This study was conducted in a facility accredited by the Association for the Assessmen and Accreditation of Laboratory Animal Care, International. The study was approved by the Institutional Animal Care and Use Committee (US Army Institute of Surgical Research, Fort Sam Houston, TX) and performed in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. Thirty-two male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; body weight, 220 ± 10 g) breathing spontaneously 100% oxygen were maintained under isoflurane (2%) anesthesia and then tracheostomized to ensure a patent airway.

Systemic Measurements

The carotid artery, femoral vein, and femoral artery were cannulated for monitoring blood pressure, infusion of dyes, and blood withdrawal, respectively. Heparinized arterial samples were collected at baseline and postresuscitation to measure hematocrit, total hemoglobin concentration, pH, lactate, and base excess (BE) (I-stat, Abbott, Chicago, IL), and plasma syndecan 1 levels. Hemoglobin O_2 saturation (SO_2) and respiratory rate (RR) were measured noninvasively (MouseOx, Starr Lifesciences, Pittsburgh, PA). Mean arterial pressure (MAP), heart rate, core temperature, SO_2, and RR were recorded continuously using a data acquisition system (Dynamic Research Evaluation Workstation, DREW, USAISR, San Antonio, TX, Model USB6218BNC, National Instruments, Austin, TX).[5] The first 1 mL of the hemorrhaged blood was collected in 3.2% citrate for coagulation assays (ROTEM, TEM Innovations GmbH, Munich, Germany).

Intravital Microscopy

The system described in detail previously[5] included a microscope (BX51WI, Olympus, Center Valey, PA), an immersion objective (Zeiss 63×, numerical aperture = 0.95), and selective filter block systems for Texas Red (TR) and fluorescein isothiocyanate (FITC) fluorescence. One optical exit of the microscope was connected to a red blood cell (RBC) velocity measuring device (Optical Doppler Velocimeter, Texas A&M, College Station, TX), a color camera (KP-D531U-S3, Hitachi, Woodbury, NY), and a monitor. The second exit was connected to a charge-coupled device camera (CoolSnap CF, Roper Scientific, NJ) with 1,392 × 1,040-pixel resolution. Blood flow and shear rate were calculated from RBC velocity and vessel diameter. Wall shear rate (WSR) was used as a measure of the levels of shear acting on the endothelial cell.

Experimental Animal Preparation

The cremaster muscle was exteriorized and positioned flat over a thermostatically controlled pedestal.[16] After exposure, the preparation was covered with a thin impermeable plastic film to minimize dehydration and gas exchange with the atmosphere. The animal platform was then placed during the microscope stage.

Fluorescently Labeled Dextran Solutions

Dextrans (Dx) of different molecular weights (Dx70, 70 kD; Dx500, 500 kD), labeled with either TR or FITC, were used to measure the space occupied by the glycocalyx as we have previously shown.[5] To determine the intact EG thickness, TR-Dx70 (10 mg/mL, Molecular Probes, Life Technologies, Carlsbad, CA) was injected at baseline, followed by FITC-Dx500 (10 mg/mL, Sigma-Aldrich, St. Louis, MO) after HS/resuscitation.

FFP Preparation

FFP is defined as plasma frozen within 6 hours to 8 hours of collection and stored at −20°C or lower for up to 1 year according to the American Association of Blood Banks. FFP was prepared by separation from whole blood collected in sterile syringe with 3.2% citrate (Baxter Healthcare Corp., Deerfield, IL) from the carotid artery of donor rats. One rat FFP unit usually consists of 5 mL. Rat FFP contains all known coagulation and anticoagulant proteins in concentrations found in normal rat blood.

Experimental Protocol

A total of 121 microvessels were studied in five experimental groups (approximately 6 rats per group). Initially, 4 to 6 microscopic fields containing venules were randomly selected. The first dye (TR-Dx70) was injected 5 min before baseline. Image sequences of microvessels (fields) using each tracer were recorded using intravital microscopy and transferred to a computer. RBC velocity was determined on-line. A set of systemic parameters and blood samples were collected during baseline coinciding with the microcirculatory data. Then, a fixed-volume hemorrhage was induced in 30 minutes (T0–T30) to a target of 40% of total blood volume (assumed as 6% of body weight) using a double-lumen catheter (Braintree Scientific, Inc., Braintree, MA) for simultaneous bleeding and infusion of 3.2% citrate followed by 30 minutes of shock (T30–T60). After 30 minutes of shock, animals were previously randomized into four treatment groups as follows: (1) HEM, hemorrhage only; (2) LR, resuscitation with LR, dose of 75 mL/kg (3× shed blood); (3) HEX, resuscitation with HEX, dose of 15 mL/kg; (4) FFP, resuscitation with fresh frozen platelet-poor plasma, dose of 15 mL/kg. Sham animals were subjected to all procedures except hemorrhage and resuscitation. One hour after the onset of resuscitation (T60–T120), animals were observed for 60 minutes (T120–T180). FITC-Dx500 was then injected, so paired recordings of the selected fields were followed by systemic measurements and collection of terminal blood samples. Shed blood was not returned to any animal.

Plasma Levels of Syndecan 1

Previously frozen plasma samples were thawed and used to analyze rat syndecan 1 using a commercial enzyme-linked immunosorbent assay kit (ABIN416453, Antibodies Online, Atlanta, GA).
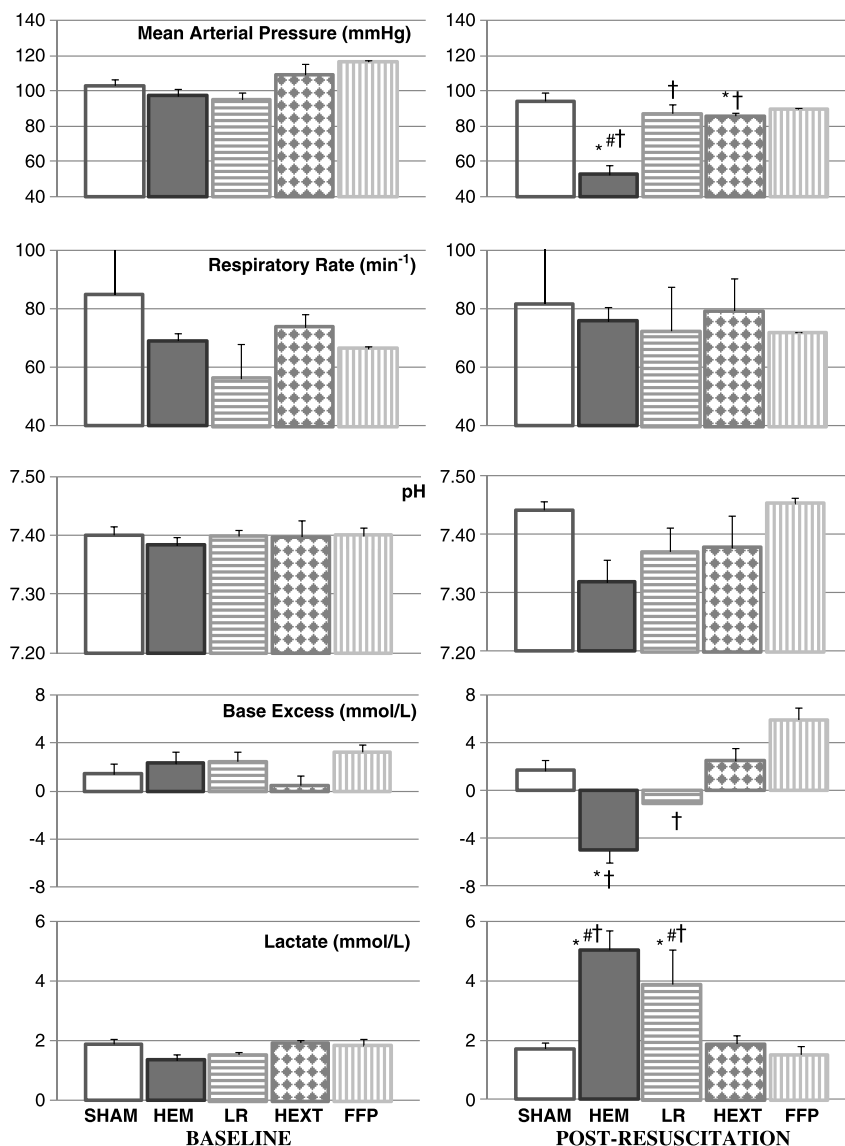
Figure 1. Representative systemic parameters for all animal groups using invasive techniques (MAP), noninvasive probes (RR), and arterial blood sampling (pH, BE, and lactate) before hemorrhage (baseline) and after different treatments (postresuscitation). Data are expressed as mean ± SEM from at least five animals per group. *Significantly different from baseline. #Significantly different from the sham group. †Significantly different from the FFP group.

Thromboelastometry

Functional assays of blood clotting were performed using ROTEM. Clotting time (in seconds), clot formation time (in seconds), α angle (in degrees), maximum clot firmness (MCF, in millimeters), and maximum clot strength (MCE, [G dynes/cm^2] / 50) were investigated in ExTEM assays, with tissue factor as the activator. In FibTEM assays, platelet function was inhibited to assess the role of functional fibrinogen in clotting. Thus, clot formation time, clotting time (CT), firmness, and strength, as well as maximum velocity (in millimeters per second) and maximum lysis were measured. Maximum clot strength was calculated as (MCF × 100) / (100 − MCF). The "platelet component" of the clot strength (ΔMCE) was calculated as $MCE_{ExTEM} - MCE_{FibTEM}$.[17,18] Maximum velocity was

obtained from the first derivative of the positive fork curve, illustrates the speed of clot initiation, and is very similar to thrombin generation curves reported in plasma.[19]

Glycocalyx Thickness Measurements

As described in detail previously,[5] we used a methodology based on two techniques to measure the EG thickness: the dye-exclusion method[20] and the image analysis method.[21] Briefly, after the completion of each dye injection, bright field images were taken of selected postcapillary venules to measure the width of the vessel (anatomic diameter). The microscope was then switched to fluorescence illumination, and a sequence of images of the fluorescent column was recorded without disturbing the image alignment with the bright field

TABLE 1. Coagulation Parameters at Baseline and Post-resuscitation

	Sham	HEM	LR	HEX	FFP
BL ExTEM CFT, s	51 ± 6	54 ± 6	52 ± 5	54 ± 7	51 ± 5
PR ExTEM CFT, s	44 ± 5	61 ± 7	74 ± 4*#	45 ± 4	46 ± 2
BL FIBTEM CFT, s	60 ± 2	57 ± 2	57 ± 1	59 ± 1	56 ± 2
PR FIBTEM CFT, s	62 ± 1	61 ± 3	54 ± 2	59 ± 1	58 ± 2
BL FIBTEM CT, s	40 ± 7	45 ± 4	43 ± 5	59 ± 16	43 ± 4
PR FIBTEM CT, s	37 ± 3	55 ± 8	65 ± 5	40 ± 4	37 ± 4
BL ExTEM MCF, mm	60 ± 2	57 ± 2	57 ± 1	59 ± 1	56 ± 2
PR ExTEM MCF, mm	62 ± 1	61 ± 3	54 ± 2	59 ± 1	58 ± 2
BL FibTEM MCF, mm	12 ± 1	10 ± 1	11 ± 0.4	10 ± 1	11 ± 0.3
PR FibTEM MCF, mm	13 ± 1	13 ± 1	9 ± 1*	7 ± 1*#	12 ± 0.4
BL FibTEM MaxV, mm/s	13 ± 2	12 ± 1	12 ± 1	14 ± 2	15 ± 1
PR FibTEM MaxV, mm/s	19 ± 3	19 ± 3	5 ± 1*†	9 ± 1*†	17 ± 2

#Significantly different from baseline.
*Significantly different from the sham group.
†Significantly different from the FFP group.
Data are expressed as mean ± SEM.
BL, baseline; CFT, clot formation time; MaxV, maximum velocity; PR, postresuscitation.

image. Later, the radial light intensity distribution was used to estimate the anatomic diameter and the width of the fluorescent column along the boundaries. The difference between the anatomic diameter and the width of the fluorescent column was used to estimate the thickness of the EG. All image processing/measurements were performed using Image-Pro Plus software (MediaCybernetics, Rockville, MD).

Statistical Analysis

SigmaPlot 12 (Systat Software, Inc., San Jose, CA) was used for the statistical analysis. Deviation from Gaussian distribution was tested using the Shapiro-Wilk test. Parametric tests were found adequate. Values are reported as mean ± SEM. Differences between before and after hemorrhage as well as among groups were analyzed by using repeated-measures analysis of variance followed by Student-Newman-Keuls test. Differences were statistically significant if $p < 0.05$.

RESULTS

Systemic Responses

The sham group was systemically stable throughout the 3-hour experimental period. Rats subjected to shock were hemorrhaged a mean volume of 25 ± 0.5 mL/kg.

After HS/resuscitation, the HEM group showed lower MAP and BE as well as higher lactate values compared to baseline and sham group ($p < 0.001$) (Fig. 1). Resuscitation with FFP showed the best overall recovery, with the lowest levels of lactate as well as the restoration of pH and BE (Fig. 1). Resuscitation with LR did not restore BE and lactate in blood to baseline levels (Fig. 1). The RR was not significantly different among the groups, averaging 71 ± 9 per minute and 79 ± 8 per minute at baseline and postresuscitation periods, respectively. Since animals were breathing 100% O_2, arterial PO_2 and SO_2 remained greater than 300 mm Hg and 99%, respectively, throughout the experiment.

There was no difference in hematocrit at baseline among the groups (mean, 40.0% ± 0.3%). The sham group did not change hematocrit before and after "resuscitation" (from 38.7% ± 1.7% to 38.8% ± 0.6%). In contrast, hemorrhage reduced the hematocrit in the HEM group to 30.4% ± 2.2% ($p < 0.001$). Resuscitation with LR and HEX significantly reduced hematocrit further to 22.4% ± 0.7% and 21.5% ± 0.6%, respectively ($p < 0.001$), compared with the sham, HEM, and FFP groups. At postresuscitation, the mean hematocrit of FFP-treated rats was significantly higher (30.0% ± 2.4%) compared with the LR and HEX groups ($p < 0.001$).

Table 1 shows a subset of the coagulation parameters for each treatment group. The LR group had a longer ExTEM CT and clotting formation time, likely due to a clot polymerization disorder (Fig. 2A), but no significant differences in firmness and velocity were observed among the groups. In contrast, there was no significant change in FibTEM CT among the groups. The HEX and LR groups showed significantly lower clot firmness and velocity than baseline and the sham group (Table 1). Moreover, clot lysis changed from 1.08% ± 0.70% to 9.01% ± 1.80% and from 0.88% ± 0.44% to 4.67% ± 1.57% in the HEX and LR groups, respectively ($p < 0.05$). These represent ninefold and fivefold increase in lysis from baseline compared with the sham and FFP group values, which indicate a trend in greater loss of clot stability. The sham and FFP groups had 16% and 53% drop in lysis, respectively, while HEM did not change. In the LR group, ΔMCE was lower than baseline ($p < 0.05$) (Fig. 2B). Resuscitation with FFP restored homeostasis in all ExTEM and FibTEM assays.

Microvascular Responses

Microvascular hemodynamics was studied in an average of 24 venules from at least five different cremaster preparations per group. Venular diameter was not significantly different among the groups, averaging 14.1 ± 0.4 μm and 13.6 ± 0.4 μm before and after HS/resuscitation, respectively. At baseline, venular RBC velocity remained unchanged among the treatment groups (pool mean, 1.76 ± 0.04 mm/s). At postresuscitation, velocity dropped 75% and 54% in HEM and LR groups, respectively ($p < 0.05$) compared with the sham group. Velocity was recovered in rats treated with HEX (to 99% of the control) and with FFP (to 63% of the control). Blood flow in the HEX group recovered to near-baseline levels (Fig. 3), whereas flow remained low in the LR and HEM groups compared with the sham group ($p < 0.05$). WSR was lower in the HEM and LR groups (vs. sham), and HEM was lower than the FFP group (Fig. 3B).

Changes in EG

The relative changes in plasma syndecan 1 compared with baseline were measured after HS/resuscitation for all treatment groups. Syndecan 1 was unchanged in sham animals throughout the experimental period and similar to baseline values (5.33 ± 1.98 μg/dL). In the FFP group, syndecan levels returned to control levels. Levels of plasma syndecan 1 were significantly elevated by HS alone (HEM group, $p < 0.02$) and by the administration of LR and HEX after HS ($p < 0.001$) compared with the sham and FFP groups (Fig. 4).

The EG thickness was not significantly different among the groups at baseline (mean, 0.425 ± 0.015 μm, n = 121, Fig. 5A).
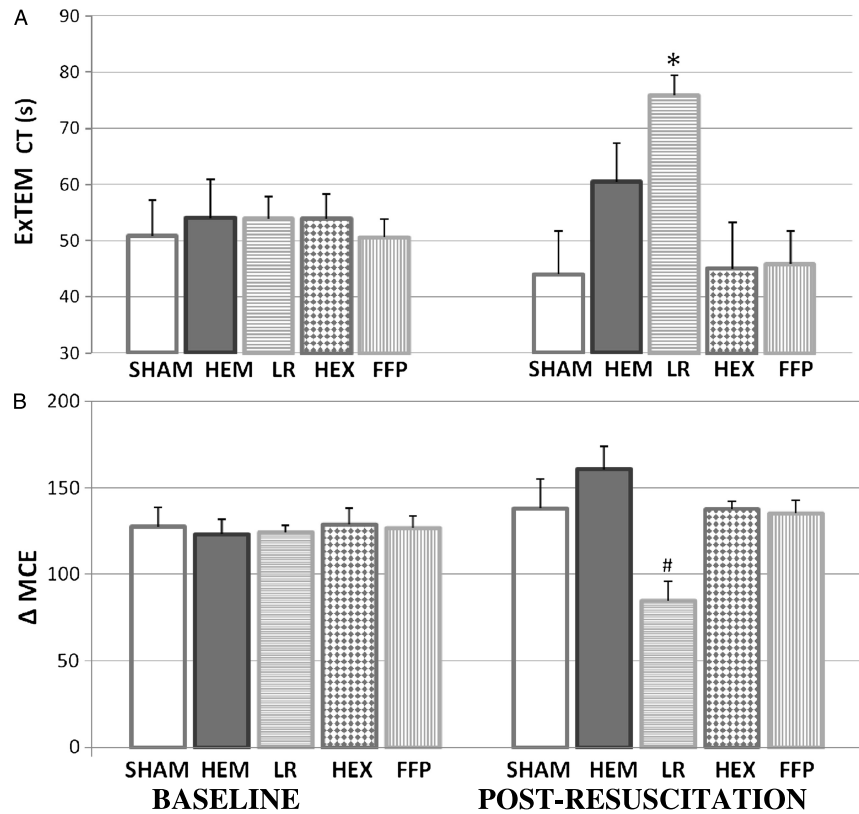
Figure 2. Effect of different resuscitation fluids on CT in the ExTEM assay (*A*) and platelet component (ΔMCE) (*B*) before and after HS. *A*, Resuscitation with LR prolonged CT compared with sham and FFP groups ($p < 0.05$), while CT in FFP-treated rats was back to normal range. *B*, Platelet component for the clot strength was markedly lower in rats resuscitated with LR ($p < 0.05$), suggestive of impaired platelet-mediated hemostasis. Data are expressed as mean ± SEM. *Significantly different from baseline. #Significantly different from the sham group.
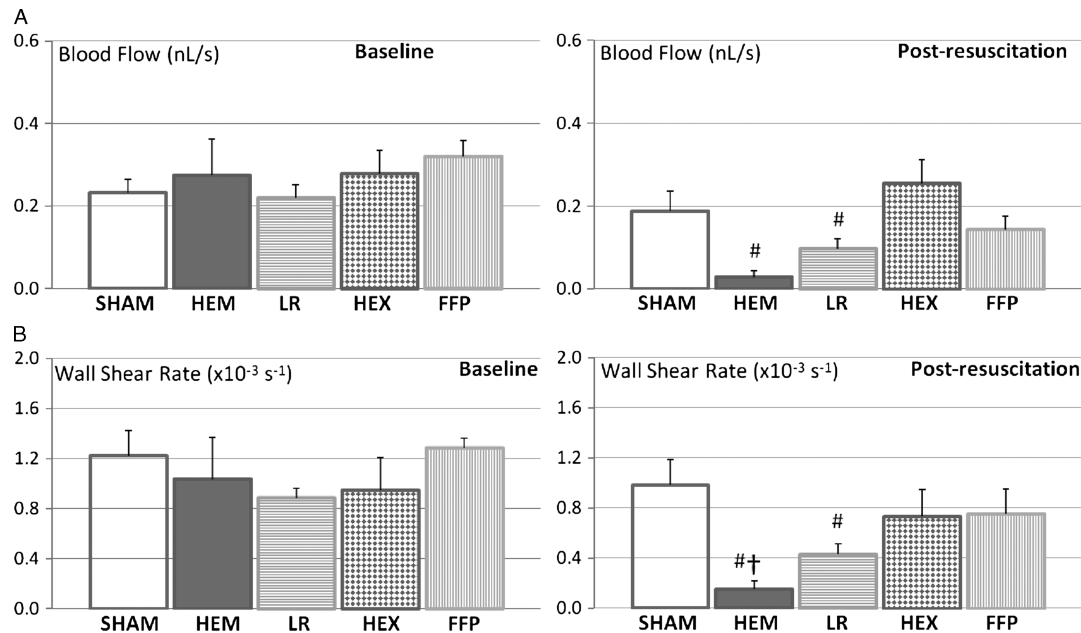
Figure 3. Blood flow and WSR of postcapillary venules from cremaster preparations. Blood flow and WSR were significantly lower in the HEM and LR groups. Sham group, 28 vessels (n = 6 rats); HEM group, 19 vessels (n = 6); LR group, 30 vessels (n = 8); HEX group, 22 vessels (n = 7); and FFP group, 22 vessels (n = 5). Data are expressed as mean ± SEM. *Significantly different from baseline. #Significantly different from the sham group.
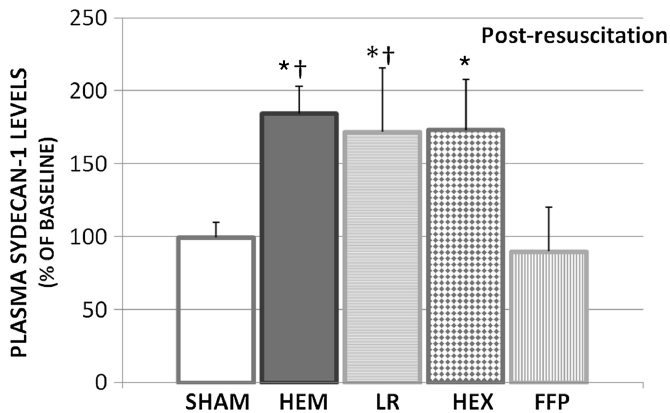
Figure 4. Levels of plasma syndecan 1 relative to baseline after HS and resuscitation treatment. Nontreated rats (HEM group) had significantly higher levels of plasma syndecan, as well as rats treated with LR and HEX (although only the LR group was significantly higher compared with the FFP group). The FFP group restored syndecan to baseline levels and therefore was lower compared with HEM group ($p < 0.001$). Data are expressed as mean ± SEM. *Significantly different from baseline. #Significantly different from the sham group. †Significantly different from the FFP group.

After resuscitation, EG thickness was significantly reduced by nearly 70% in the HEM, LR, and HEX groups to a pooled mean of 0.132 ± 0.010 μm (Fig. 5*B*). However, FFP-treated rats recovered the EG thickness (0.532 ± 0.060 μm) 1 hour after resuscitation (no significant difference from baseline and the sham group).

DISCUSSION

The combined evaluation of microcirculation and systemic physiologic parameters, as presented here, is important but less often performed when comparing resuscitation fluids. In vivo glycocalyx changes were investigated after HS/resuscitation using the cremaster muscle model. The microvasculature of this skeletal muscle is well described and has been extensively studied, providing information on the pathophysiologic aspects of HS.[5,22,23]

There was a significant drop in MAP, BE, and pH as well as a rise in lactate levels due to the development of metabolic acidosis and widespread tissue ischemia/hypoxia caused by blood loss.[24] Rats that received LR had increased systemic MAP but did not sustain peripheral tissue perfusion, and therefore, pH, BE, and lactate returned to the shock level. Conversely, the infusion of HEX and FFP restored cardiovascular function and reversed metabolic acidosis.

The equal hematocrit changes in response to LR and HEX indicate that there was an equal volume expansion in response to the 3-to-1 volume of LR to the shed blood volume as to the HEX volume. The LR distributes to the entire extracellular space, with only one third of the LR remaining in the vascular space. The hyperoncoticity of the HEX resulted in the recruitment of fluid from the interstitial space to cause equal hemodilution as the LR despite less resuscitation volume than the shed blood volume being given. FFP resulted in less

hemodilution since it caused a smaller reduction in hematocrit compared with HEX.

Military units operating in isolated, dispersed areas have been known to carry up to 4 U of blood products on helicopters, including FFP and RBCs, to administer as an initial resuscitation fluid to treat casualties far forward.[25] Four units of FFP is equivalent to a dose of 15 mL/kg. Military 2003 and 2006 Tactical Combat Casualty Care guidelines for casualties in HS recommend that no more than 1 L (approximately 14 mL/kg for a 70-kg man) of HEX can be administered in the patient. An equivalent colloid dose of 15 mL/kg ensures concomitant volume expansion and hypotensive resuscitation. Hetastarch is recommended over crystalloids because of its longer maintenance in the intravascular space, precluding the need for additional fluid administration in cases of delayed evacuation.[26]

The drop in FibTEM firmness for the LR and HEX groups is indicative of decreased fibrinogen concentration and/or platelet number or function (in assay) and fibrin polymerization disorders or low activity of factor XIII.[18] The impact of resuscitation fluids on clot firmness, as presented here, is in keeping with previous investigations showing reduced fibrinogen levels in response to HS.[27,28] The fibrinogen and other coagulation factors were diluted with the LR and HEX resuscitation[29] as demonstrated with low FibTEM firmness. This contrasts with no change in ExTEM firmness, suggesting
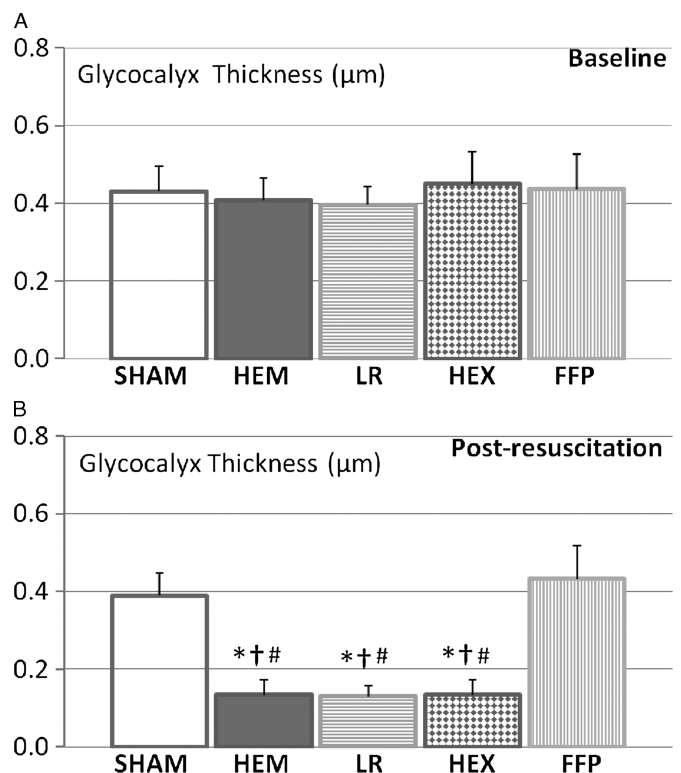
Figure 5. EG thickness in postcapillary venules from cremaster preparations. See Figure 3 for definitions, number of animals, and number of vessels per group. Data are expressed as mean ± SEM. *Significantly different from baseline. #Significantly different from the sham group. †Significantly different from the FFP group).

that platelets were probably recruited from the reticuloendothelial system to counteract the hemorrhage and hemodilution.[29] Conversely, resuscitation with HEX in coagulopathic rabbits decreased the firmness in platelet-rich blood.[30] Discrepancies in the HEX infusion dose and the severity of hemorrhage model can account for the difference in outcomes. The velocity is similar to thrombin generation curves reported in plasma,[19] and a drop in FibTEM velocity for the LR and HEX groups corroborates with findings showing that imbalances in thrombin generation depend on fibrinogen concentration, even in the presence of normal platelet count or function.[3,18,31]

High levels of heparan sulfates and heparin-like proteoglycans secondary to EG shedding may play a role in modulating fibrinolytic events.[32] The significant increase of FibTEM lysis in the HEX group ($p < 0.001$) at postresuscitation suggests a low-grade fibrinolysis when platelets are inhibited, as demonstrated previously.[3,17]

The prolonged CT and the drop in ΔMCE found in the LR group were possibly caused by a dilution effect on the platelets after tissue factor activation. Changes in CT and ΔMCE were not statistically significant in the HEX group likely because the calcium included in the formulation counteracted the effect of hetastarch on coagulation interfering with platelet function. Our data suggest that clotting factors and fibrinogen in the FFP improved coagulation deficiencies seen with LR or HEX, despite some hemodilution by FFP administration.

Viscous solutions such as HEX may improve microvascular flow and functional capillary density.[12,33] The increase in RBC velocity and blood flow seen after HEX administration is caused by HEX hyperoncoticity that causes severe hemodilution and decrease in flow resistance.[2] EG thickness depends on the plasma composition and on the local hemodynamic conditions.[34] Despite systemic and microhemodynamic recovery, LR and HEX did not provide any glycocalyx restoration. A meta-analysis of studies that used protein-free intravenously administered fluids for hemodilution showed that these fluids led to a loss of EG-adsorbed proteins and proteoglycans.[35] This could help explain our findings that the LR and HEX groups had the lowest glycocalyx thickness, whereas the FFP group showed the best EG restoration. Previously, a 14% to 20% drop in vascular resistance was seen after enzymatic removal of the glycocalyx with heparinase.[7,35] This evidence ties with our observation of low EG thickness and increased blood flow and velocity in the HEX group. The reinstitution of EG structure may play an essential role in tissue perfusion after shock by increasing resistance and enhancing shear stress interaction with the endothelium.

It has been demonstrated that the EG is present throughout the microcirculation. Our group has recently obtained direct in vivo data showing approximately the same glycocalyx thickness reduction in venules from skeletal muscle and mesentery after HS.[5] Lipowsky et al.[13] reported similar glycocalyx thickness in the arterioles and venules but larger in the capillaries of rat mesentery but analogous reductions in response to Formyl-Methionyl-Leucyl-Phenylalanine among the arterioles, capillaries, and venules. Savery and Damiano[36] found comparable results between the arterioles and venules of mouse cremaster after shedding of the glycocalyx with hyaluronidase, which supports the existence of a similar response among the microvessels after shedding.

Attempts to reconstitute the EG after its degradation have been verified in vivo with doxycycline,[14] preventing GAGs release and collagen degradation,[37] which also disturbs coagulation. In addition, inhibition of heparitinase or infusion of GAGs demonstrated efficacy in restoring EG and inhibiting neuthrophil adhesion.[38] In our study, restoration of EG thickness was achieved after FFP administration, likely owing to blood-borne proteins and GAGs in physiologic plasma concentrations.

The protection or restoration of a previously damaged glycocalyx has been considered a promising therapeutic target in acute critical care settings: EG may play a role in vascular hemostasis.[10,12] Subramanian et al.[39] found that thrombin accelerates the shedding of the syndecan 1 and 4 ectodomains from cultured endothelial cell, via G protein-coupled. This may explain EG shedding during hemorrhage due to an increase in thrombin generation to counteract hemorrhage. In a prospective study in trauma patients, the rise in catecholamines was associated with severe endothelial cell dysfunction, large release of tissue plasminogen activator, and increase in plasma syndecan 1, presumably owing to EG shedding.[10]

This study showed that fully repaired EG thickness was achieved with the administration of plasma after HS. Changes in EG thickness were reported in mesenteric venules using electron microscopy after a severe, prolonged fixed-pressure model of HS treated with large volume of FFP.[40] In our protocol, the glycocalyx restoration was complete following infusion of a much smaller FFP volume than previously reported.

CONCLUSION

In conclusion, this work demonstrated reconstitution of venular EG and microhemodynamics as well as improvement in vascular hemostasis by a small-volume resuscitation with FFP in rats subjected to HS, in contrast to resuscitation with LR and HEX, which were unable to achieve EG and coagulation repairs. The drop in clot firmness for the LR and HEX groups is indicative of decreased fibrinogen concentration, possibly due to blood dilution by LR and HEX. In addition, low fibrinogen concentration may lead to imbalances in thrombin generation, which supports our findings that FibTEM velocity is decreased in those groups. FFP-treated rats displayed restoration of coagulation function. The efficacy of FFP to reinstate perfusion may be related to the reconstitution of EG structure, volume expansion, and restoration of total protein levels. The data support the concept of cardiovascular and microvascular stabilization by infused plasma, in which the increase in microvascular perfusion associated with repaired EG is essential for an optimal resuscitation strategy.

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